muscle-related gene signature were not, however, seen in ageing Str/ort mice and these differences confirmed by RT-qPCR for Myf6 and Myh1. Comparison of Str/ort mice at these same ages, during which OA develops, showed 279 differentially regulated genes (47 up, 232 down) and these were mainly centred on the signalling via the NFkB complex.
To determine the gene expression patterns related to OA susceptibility, 8wk-old CBA and Str/ort mice AC were compared. This showed up-regulation of 139 genes (none down-regulated), including Htra1 and TIMP1, which was again centred primarily on NFkB pathway signalling. Immunolabelling of AC sections showed that the NfkB-p65 protein was more highly expressed in chondrocytes of 8wk-old Str/ort than in aged-matched CBA mice.
To define gene profiles specific to early OA, AC from 8 and 18wks old Str/ort mice was compared. This showed 113 down-regulated and only 2 up-regulated genes and pathway analysis showed that these were again centred on the NFkB pathway. Interestingly, no genes were differentially regulated between 18 and 40wks in Str/ort mice, suggesting that similar processes are taking place in both early and late-stage OA.
Conclusions: We find that AC chondrocytes express skeletal muscle-related genes in young mice that are lost during normal healthy ageing but retained during OA. In addition, our data support the involvement of NFkB pathway signalling in susceptibility of AC to spontaneous OA and to OA progression. This study highlights these molecular processes as possible markers of OA and targets for slowing OA development.

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GENERATION OF AN INDUCIBLE CARTILAGE SPECIFIC DELETER USING HUMAN AGGRECAN ENHANCER/PROMOTER THAT IS TRACKABLE IN VIVO USING LUCIFERASE

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Purpose: To generate a chondrocyte-specific deleter which can be visualised in vivo. We needed an improvement on the collagen type II which is significantly reduced in adult articular cartilage and expresses in kidney during development
Methods: We have utilized a transgenic approach where we have used the human aggrecan enhancer/promoter to drive inducible Cre recombinase (Cre-ERT2) followed by an IRES luciferase forming a bicistronic mRNA in transgenic mice.
Results: The expression and efficiency of the inducible cre recombinase was tested by examining X-gal staining of tissues from embryos as well as adult in double transgenic with Rosa 26R mice. Cre recombinase was induced by tamoxifen, at different time points during development and postnatally. X-gal staining was observed in growth plate and articular cartilage as well as the fibrocartilage of meniscus, trachea, and intervertebral discs reproducing the pattern of endogenous aggrecan gene expression.
In addition to this mouse being an efficient deleter, the presence of luciferase allows the visualization of aggrecan expression in vivo. This has been tested before or after the induction of osteoarthritis through destabilisation of the medial meniscus ligament up to eight weeks post surgery.
Conclusions: The aggrecan-CreERT2 will help us determine genes involvement in the integrity of the cartilage and visualize this complex in osteoarthritis

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DEFICIENCY OF NFAT1 TRANSCRIPTION FACTOR CAUSES OSTEOARTHRITIS WITH ALTERATIONS IN ARTICULAR CARTILAGE AND SUBCHONDRAL BONE IN ADULT MICE

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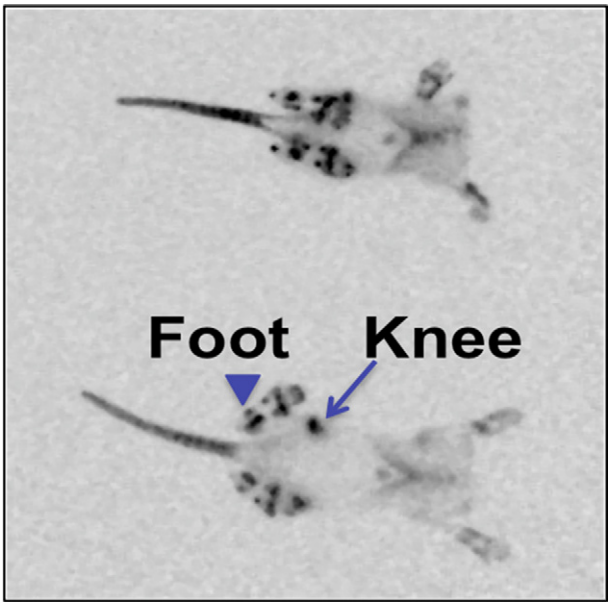
Purpose: One of the barriers to progress in OA research is the difficulty in obtaining human joint tissue samples at an early stage of OA that permits studies of the mechanisms for initiation of OA. Many animal models have been developed to explore the mechanisms of human OA. However, surgically- or chemically-induced OA models often display rapidly progressive joint lesions and may not be suitable for studying the etiopathogenesis of more slowly progressive non-traumatic OA in humans. Murine models of OA developed by spontaneous or genetically-induced



mutations of genes for cartilage matrix proteins or growth factors are often accompanied by developmental defects in the skeletal system (e.g., mice harboring mutations in aggrecan or type-II collagen). Notably, these mouse models usually do not show subchondral bone changes, an important pathogenetic feature of human OA. Therefore, there is a crucial need for animal models that mimic the pathologic characteristics of idiopathic human OA to study the mechanisms for initiation and progression of OA.
Methods: We examined whether deletion of Nfat1, a transcription factor previously reported as a regulator of the expression of cytokine genes during the immune response, would alter the expression of specific proinflammatory cytokines and display osteoarthritic changes in articular tissues of mice.
Results: The results revealed that deletion of Nfat1 transcription factor in mice caused classic OA changes, including alterations in articular cartilage and subchondral bone that mimic those in human OA. Nfat1-deficient mice exhibited normal skeletal development but displayed loss of type-II collagen and aggrecan in young adult articular cartilage of load-bearing joints. These early changes were followed by articular chondrocyte proliferation/clustering, thickening of subchondral bone, slow progression of articular surface destruction, and formation of chondro-osteophytes. Overexpression of specific matrix-degrading proteinases and proinflammatory cytokines was observed in Nfat1-deficient articular cartilage and synovium. We identified Nfat1 binding sites in the promoters of the genes for mouse and human interleukin-1β, tumor necrosis factor-α, matrix metalloproteinase-13 (MMP13), and a disintegrin and metalloproteinase with thrombospondin motifs-4,5 (ADAMTS-4,5), suggesting that Nfat1 may be an upstream regulator of these catabolic molecules that degrade articular cartilage.
Conclusions: These novel findings suggest that Nfat1-deficient mouse may serve as a more suitable surrogate of human OA than murine OA models that do not exhibit subchondral bone changes. Because Nfat1 regulates multiple matrix-degrading proteinases and proinflammatory cytokines in articular tissues, anti-OA agents that target Nfat1 could be more effective than drug candidates that target a single catabolic molecule.

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EARLY INTERVENTION TO PREVENT CARTILAGE DEGENERATION BY ADMINISTRATION OF ANTI-VEGF ANTIBODY IN RABBIT MODEL

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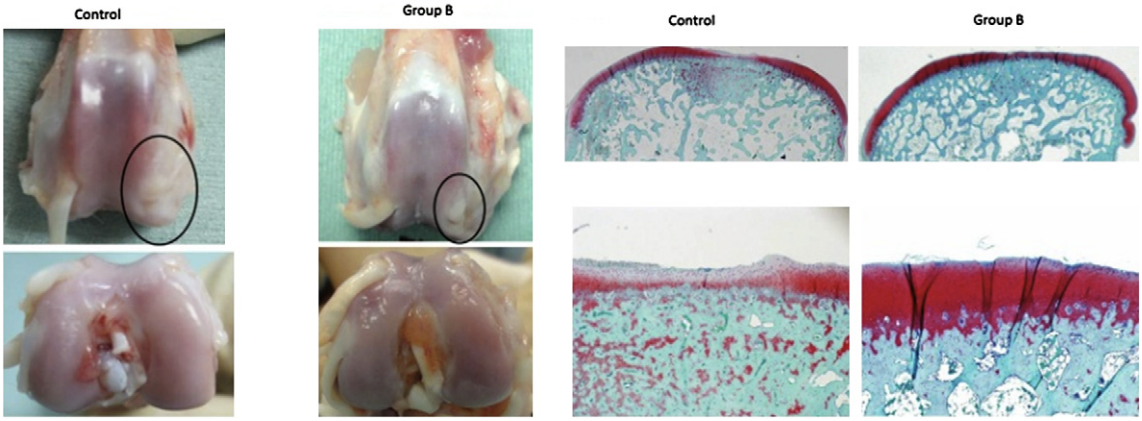
Purpose: We have previously constructed and transplanted scaffold-free tissue-engineered cartilage into an osteochondral defect (Tissue Eng Part A 2008;14:1183-1193), and confirmed that reparative cells derived from



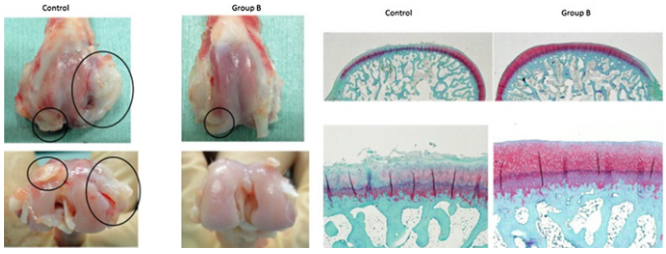
bone marrow acquired properties of anti-angiogenesis to achieve good restorative effects of articular cartilage (Tissue Eng Part A 2008;14:1225-1235.). Then we reported that intravenous administration of bevacizumab, a humanized monoclonal anti-VEGF antibody contributes to the repair of articular cartilage in an osteochondral defect model (Arthritis research and therapy 2010,12:R178.). The object of this study was to investigate whether cartilage degeneration is prevented following intravenous administration of bevacizumab in the rabbit model of anterior cruciate ligament transection (ACLT).

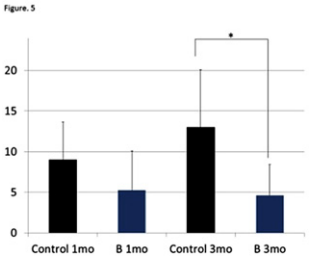
Methods: ACLT rabbits were classified into two groups: Group B, administration of bevacizumab (n=15; 100 mg intravenous injection on the day of 1 and 3 weeks after surgery); and the Control (n=16).

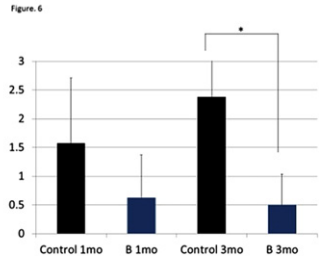
Osteophyte formation was evaluated macroscopically and OA repair sites were also evaluated using OARSI modified Mankin score.

Results: One month after ACLT, macroscopic evaluation of both groups showed some parts of joints included osteophyte formation and almost smooth joints surface of the articular cartilage (Figure.1). However histologic assessment demonstrated that articular cartilage was recognized loss of staining in Control. Group B was tended to retain staining (Figure.2).

3 months after ACLT, macroscopic evaluation of the Control showed marked progression of arthritis and osteophyte formation. Group B still showed smooth joint surfaces in the most regions of the articular cartilage and osteophyte formation was restrained (Figure.3, 4).







OARSI histological score was used to evaluate the quality of the repair tissue (Figure.5). Osteophyte formation score 3 month after ACLT in B group was significantly lower than control (Figure.6).

Conclusions: The early intervention of intravenous administration of bevacizumab would be useful to prevent the post-traumatic osteoarthritis.

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DIFFERENCES IN STRUCTURAL AND PAIN PHENOTYPES BETWEEN MONOIODOACETAE AND MENISCAL TRANSECTION MODELS OF OSTEOARTHRITIS

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Purpose: To compare pathology in two animal models of osteoarthritis at several time points after the induction of arthritis and to investigate pain behaviour over the course of the experiment.

Methods: Meniscal transection (MNX) arthritis was induced by transection of the meniscus in 180g male Sprague Dawley rats. Controls were animals subjected to sham surgery in which the collateral ligament was removed but the meniscus was left intact. Monosodium iodoacetate(MIA) arthritis was induced by the intra-articular injection of 1mg of sodium monoiodacetate dissolved in 50µl sterile saline. Separate groups of animals injected with saline were controls. Groups of animals (n=8) were sacrificed at 14, 35 and 45 days. Pain behaviour was monitored by differential weight bearing (g) and paw withdrawal thresholds (g). Pathology was scored for synovial inflammation (0-3), chondropathy (0-15), osteophytes (0-3) and the number of osteochondral channels /mm length of cartilage were counted. Data are means (95% CI).

Results: Inflammation in the synovium remained constant over time. At day 49, MNX- treated knees had greater inflammation 1.7 (1.2-2.1) than Sham controls 0.2 (0.02-0.4) $p<0.01$, and MIA- treated knees were more inflamed 0.6 (0.2-0.8) than saline-injected controls 0.0 (0-0). MNX- treated knees were more inflamed than MIA -treated knees, $p> 0.01$. Osteophyte scores were greater at all time points in MNX- treated knees when compared to MIA -treated knees. At day 49 osteophytes scores were greater in MNX- treated knees 2.8 (2.6-3.0) than sham 0.23 (0.02-0.4), $p<0.01$, and greater in MIA-treated knees 1.0 (0.5-1.4) than in saline-injected controls 0 (0-0), $p<0.01$. Osteophyte scores were greater in MNX-treated knees than in MIA-treated knees ($p<0.01$). Chondropathy scores increased in both treatment groups with time and did not differ between MNX-treated knees and MIA-treated knees. At day 49 MNX-treated knees had higher osteophyte scores 12.7 (11.1-14.3) than sham controls 2.8 (0.8-4.8), $p<0.01$. Osteophyte scores in MIA-treated knees 9.7 (7.5-11.8) were higher than in saline controls 0 (0-0), $p<0.01$. At day 49 MNX-treated animals displayed greater numbers of channels crossing the osteochondral junction 0.25 (0.1-0.4) channels/mm compared to sham controls 0.01 (0.03-0.1), MIA-treated knees 0.1 (0.09-0.1) and saline controls 0.04 (0.01-0.2). MNX- treated animals displayed greater weight bearing asymmetry